EVALUATION OF SERUM VITAMIN B12 LEVELS IN TYPE 2 DIABETES PATIENTS ON METFORMIN THERAPY ATTENDING A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND

Type 2 Diabetes Mellitus (T2DM) is an endocrine disorder and a heterogeneous group of syndromes characterised by an elevation of fasting blood glucose that is caused by a relative or absolute deficiency in insulin. Serum vitamin B12 levels have been reported to be inversely associated with the dose and duration of metformin use. Vitamin B12 deficiency is a treatable condition. However, there is insufficient data regarding prevalence of vitamin B12 deficiency in the South Indian population. Hence, the study was undertaken to evaluate serum vitamin B12 levels in T2DM on metformin therapy.

MATERIALS AND METHODS

A total of 100 T2DM patients attending Outpatient Department (OPD) or admitted under Department of General Medicine of KIMS, Hubli, during the time period of 2 years were taken for study considering the inclusion and exclusion criteria. Qualifying patients underwent detailed history, clinical examination, routine investigation and vitamin B12 estimation.

RESULTS

Majority were in the age group of 45-59 years making 42% of the total. The age group in most patients having vitamin B12 deficiency was >60 years. Males had more vitamin B12 deficiency forming 54.5%. Vitamin B12 deficiency was more in hypertensive group accounting for 71.4%, which was statistically significant. Most of the vitamin B12 deficiency patients had diabetes duration >10 years. Most of the vitamin B12 deficient patients were taking metformin therapy for longer years (≥10 years) and in higher dose (>2 g/day). Most of the vitamin B12 deficient diabetes patients were overweight forming 52.5% of the total.

CONCLUSION

Longer duration of diabetes increases the risk of developing vitamin B12 deficiency on metformin therapy. The higher dose of metformin intake had inverse relation with vitamin B12 levels. Longer duration of metformin intake causes vitamin B12 deficiency and hence should be screened for vitamin B12 deficiency and can be supplemented with vitamin B12.

KEYWORDS

Vitamin B12, T2DM, Metformin.

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BACKGROUND

T2DM is one of the most common health problems facing mankind and International Diabetes Federation (IDF) estimated that in 2015 that 415 million people had diabetes worldwide.[1] T2DM is the predominant form of diabetes worldwide accounting for 90% of cases globally.[2]

Metformin has been used to combat high blood glucose and the main site of metformin action is thought to be in the liver where the drug suppresses glucose production and promotes glucose uptake by tissues via the glucose transporters and inhibits hepatic gluconeogenesis.[3] Metformin has shown that these effects are mediated by the activation of an upstream kinase, liver kinase B1 (LKB-1), resulting in its inactivation and thus the down regulation of transcriptional events that promote synthesis of gluconeogenesis enzymes.[4] Metformin decreases hyperglycaemia primarily by suppressing glucose production by liver (hepatic gluconeogenesis).[5] The average person with T2DM has three times the normal rate of gluconeogenesis. Metformin treatment reduces this by over one third.[6] There are numerous additional actions of metformin such as alterations to small intestine, glucose metabolism and small bowel absorption that alter the absorption profiles of some vitamins.[7]

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Vitamin B12, also referred to as cobalamin is thought to play a large role in the formation of blood as well as key roles in brain and nerve function.[8] Serum vitamin B12 levels have been reported to be inversely associated with the dose and duration of metformin use.[9,10,11] These studies reported that an average of 10 to 30% of patients exhibited malabsorptive vitamin B12 deficiency. Unfortunately, the symptoms of diabetic neuropathy overlap with the paraesthesia, impaired vibration sense and impaired proprioception associated with B12 deficiency.[12,13]

Vitamin B12 deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels, only slightly lower than normal, a range of symptoms such as fatigue, depression and poor memory maybe experienced.[14] Vitamin B12 deficiency can also cause symptoms of mania and psychosis.[15,16] Although, decrease in vitamin B12 levels following metformin use typically starts as early as the fourth month.[17] Clinical features of vitamin B12 deficiency become apparent by 5 years owing to the large body stores in the liver that are not quickly depleted.[11]

MATERIALS AND METHODS

Objectives of Study-

1. To study the association of vitamin B12 levels with various risk factors of T2DM patients treated with metformin.
2. To study the association of vitamin B12 levels with respect to the duration of metformin therapy.
3. To study the association of vitamin B12 levels with respect to the dose of metformin therapy.

Study Type- Single-centred cross-sectional study.
Study Duration- October 2014 - August 2016.
Study Design- Patients attending OPD or admitted under Department of General Medicine of Karnataka Institute of Medical Sciences (KIMS), Hubli. As it was time-bound study, subjects were selected considering inclusion and exclusion criteria and all the samples collected during the study period were included with a number of subjects restricted to 100.

Inclusion Criteria

T2DM patients defined as per ADA recommendation on metformin therapy for more than 3 months, both males and females aged ≥18 years.

Exclusion Criteria

Patients with type 1 diabetes, patients who had pernicious anaemia, megaloblastic anaemia, peripheral neuropathy, pregnant women, decreased renal function (serum creatinine levels >2 mg/dL for men and >1.8 mg/dL for women), gastrectomy, colectomy, prior bariatric surgery, thyroid disorders. Medication history was evaluated by a dietary supplement questionnaire, which includes over-the-counter multivitamins, calcium supplements, vitamin B12 injections, antiepileptics, fibric acid derivatives, thiazolidinediones and proton pump inhibitors.

Method- Patients demographic profile and risk factors were recorded for correlation. Routine blood investigations, vitamin B12, fasting blood sugar, postprandial blood sugar, fasting lipid profile samples were collected and were determined enzymatically by using an auto-analyser. In the present study, vitamin B12 concentrations of ≤148 pmol/L will be considered as deficient and vitamin B12 concentrations >148 pmol/L will be considered as normal.

RESULTS

A total of 100 T2DM subjects on metformin therapy were included in the present study.

Characteristics	
Mean SD* of age in years	52.4 (12.7)
Age Categories	
<45 years	24
45-59 years	42
≥60 years	34
Gender	
Male	66
Female	34
History of Alcohol Intake	
Present	50
Absent	50
History of Smoking/Tobacco Chewing	
Present	43
Absent	57

Table 1. Sociodemographic Characteristics of the Study Participants (N=100)

*SD=Standard deviation.

Characteristic	Mean (SD)
Duration of diabetes mellitus in years	4.8 (2.8)
Dosage of metformin in grams	2.1 (0.7)
FBS in mg/dL	159.8 (23.6)
PPBS in mg/dL	222.7 (36.4)
HbA1c in %	7.6 (0.8)

Table 2. Characteristics Related to Diabetes Mellitus among the Study Participants (N=100)

*SD=Standard deviation.

Factor	Vitamin B12 Levels		T Statistic*	P Value
	Normal n (%)	Deficient n(%)		
Age in years	47.2 (1.6)	57.3 (12.2)	4.34	<0.001
Duration of diabetes mellitus in years	3.1 (1.7)	6.3 (2.7)	7.21	<0.001
Dosage of metformin in grams	1.6 (0.6)	2.5 (0.5)	7.51	<0.001
FBS in mg/dL	154.2 (24.3)	165.1 (21.9)	2.35	<0.001
PPBS in mg/dL	210.9 (39.9)	234.0 (28.7)	3.32	<0.001
HbA1c in %	7.3 (0.7)	7.9 (0.9)	3.60	<0.001

Table 3. Correlation of Mean of Clinical Variables with Vitamin B12 Levels

*Unpaired t-test, numbers in parentheses indicate standard deviation.

Factor	Vitamin B12 Levels		Test Statistic*	P Value
	Normal n (%)	Deficient n (%)		
Age				
<45 years	16 (66.7)	8 (33.3)	13.6	0.001
45-59 years	25 (59.5)	17 (40.5)		
≥60 years	8 (23.5)	26 (76.4)		
Gender				
Male	30 (45.4)	36 (54.5)	0.97	0.32
Female	19 (55.9)	15 (44.1)		
History of Alcohol Intake				
Present	20 (40)	30 (60)	3.24	0.07
Absent	29 (58)	21 (42)		
History of Smoking				
Present	19 (44.2)	24 (55.8)	0.69	0.40
Absent	30 (52.6)	27 (47.3)		
Hypertension				
Present	6 (28.6)	15 (71.4)	4.44	0.03
Absent	43 (54.4)	36 (45.6)		
Anaemia				
Present	28 (43.7)	36 (56.3)	1.96	0.16
Absent	21 (58.3)	15 (41.7)		
Duration of Diabetes				
Less than 4 years	32 (84.2)	6 (15.8)	31.8	<0.001
4 to 9 years	16 (30.7)	36 (69.2)		
10 or more years	1 (10)	9 (90)		
Metformin Dosage				
≤1 gram	16 (94.1)	1 (5.9)	33.7	<0.001
1.5 to 2 grams	28 (59.6)	19 (40.4)		
≥2.5 grams	5 (13.9)	31 (86.1)		
FBS				
Controlled	7 (77.8)	2 (22.2)	3.27	0.07
Uncontrolled	42 (46.1)	49 (53.9)		
PPBS				
Controlled	12 (92.3)	1 (7.7)	11.21	0.001
Uncontrolled	37 (42.5)	50 (57.5)		
HbA1c				
Controlled	13 (68.4)	6 (31.6)	3.54	0.06
Uncontrolled	36 (44.4)	45 (55.6)		
BMI				
Normal	2 (100)	0 (0)		0.52
Overweight	28 (47.5)	31 (52.5)		
Obese	19 (48.7)	20 (51.3)		
TG Level				
Normal	16 (59.3)	11 (40.7)	1.55	0.21
High	33 (45.2)	40 (54.8)		

Table 4. Factors Affecting Vitamin B12 Levels among Diabetes Patients in the Study

*Chi-square test.

DISCUSSION

Metformin is a key treatment option and the most frequently prescribed first line therapy for individuals with T2DM. Absorption of vitamin B12 is through the formation of vitamin B12 intrinsic factor complex and uptake by ileal cell-surface receptors. Metformin gives a positive charge to the surface of the membrane, which acts to displace divalent cation and thus interferes with process of vitamin B12 absorption.[18] Hence, intestinal absorption of vitamin B12 is often decreased during chronic metformin therapy.

Most of the previous studies like P. Flipsen et al[12] have compared the vitamin B12 levels in diabetes patients who are taking metformin and not taking metformin therapy. The prevalence of vitamin B12 deficiency was in the range of 15-30%. In our study, 23% of T2DM patients on metformin therapy were found to have vitamin B12 deficiency.

In our study, largest numbers of patients belonged to the age group of 45-59 years. Patients with vitamin B12 deficiency were of higher age group >60 years forming 76.4% compared to normal level individuals who were of age group <45 years forming 66.7% with a statistical significance of P value 0.001.

Among 100 patients, 50 had history of alcohol consumption. Among females, only 3 had history of alcohol consumption and among males 47 had history of alcohol consumption reflecting alcohol consumption was more in males. Patients who had history of alcohol intake had more vitamin B12 deficiency accounting for 60% though not statistically significant. Alcohol consumption and smoking has been increasing in female gender in the recent past, however, our study did not show any female preponderance.

Among 100 patients, 57 had history of smoking/tobacco chewing, and the rest 43, there was no such history. Among females, none had history of smoking/tobacco chewing. Male patients had vitamin B12 deficiency, more in smoking/tobacco chewing group accounting for 55.8%, though not statistically significant.

Among 100 study participants, 52 participants were in the group of 4 to 9.91 years of duration of diabetes forming 52%. This group formed the majority among study participants. Ninety percent vitamin B12 deficiency patients were having diabetes for longer duration, more than 10 years when compared to 10% of normal patients. Longer duration of diabetes is significantly associated with vitamin B12 deficiency with P value <0.001.

Among 100 study participants, 52 participants were in the group of 4 to 9.91 years (months expressed in years) of duration of diabetes forming 52%. This group formed the majority among study participants. Most of the normal patients had diabetes <4 years forming 84.2% of the total. 90% vitamin B12 deficiency patients were having diabetes for longer duration >10 years when compared to 10% of normal patients. Longer duration of diabetes is significantly associated with vitamin B12 deficiency with P value <0.001.

Vitamin B12 deficiency was more in group where anaemia was present accounting for 56.3% though not statistically significant. Five participants had Mean Corpuscular Volume (MCV) >100 femtolitre (fL). The classic form of anaemia due to vitamin B12 deficiency is megaloblastic anaemia (MCV >100 fL).[13] However, the observed mean MCV level in our subjects with vitamin B12 deficiency was not over 100 fL and the prevalence of megaloblastic anaemia was about 5%. There were no differences in the mean MCV between the groups with and without vitamin B12 deficiency. Anaemia of our patients was most likely to have a multifactorial cause. Though megaloblastic anaemia is widely regarded to have an increased MCV, previous reports have indicated that up to 30% of vitamin B12 responsive disorders have normal MCVs.[19,20]

Most of the vitamin B12 deficiency patients had uncontrolled fasting blood glucose forming 53.9% of the total when compared to non-deficient patients though not statistically significant.

Most of the vitamin B12 deficiency patients had significant uncontrolled postprandial blood glucose forming 57.5% of the total when compared to non-deficient patients with P value of 0.001. Above studies did not show any correlation between low vitamin B12 levels and high FBS, but correlates with high PPBS levels.

Although, many patients had good control of HbA1c, but the HbA1c was mildly higher in the deficiency group when compared to the normal group. Most of the vitamin B12 deficiency patients had uncontrolled HbA1c forming 55.6% of the total though not statistically significant.

Highest numbers of cases were in the group of 1.5-2 grams of daily metformin intake forming 47% of the total in our study. The dose of metformin intake was higher in vitamin B12 deficient groups (>2.5 g daily) when compared to normal vitamin B12 individuals. The dose of metformin had inverse correlation with B12 levels and the difference was statistically significant with p value <0.001. Although, the mechanism through, which high dose of metformin causes vitamin B12 deficiency is presently not well understood. Our observation probably indicates that there is heightened inhibition of vitamin B12 absorption, which could cause rapid depletion of the liver store of vitamin B12 in patients taking high dose of metformin. Our study had mean dosage of metformin 2.5 ± 0.5 grams/day in vitamin B12 deficient patients.

In our study, the duration of metformin intake was higher in vitamin B12 deficient group >10 years when compared to normal vitamin B12 group, which was statistically significant with a p value <0.001. When comparing patients on metformin for less than <4 years with those on metformin for more than 10 years in B12 deficient patients, results were statistically significant with p-value <0.001.

Among diabetes patients with high triglyceride levels (>150 mg/dL), 54.8% had vitamin B12 deficiency though not statistically significant in our study.

Our study limitations were the smaller sample size, non-inclusion of age-matched control group and also we had a small number of female participants in comparison to males. This limitation may cause our inability to precisely evaluate the role of vitamin B12 in sexes.

One study, which aimed to evaluate the vitamin B12 status of elderly population as in New Zealand (age >65) study claimed there is a 35.54% prevalence of lowered serum vitamin B12 level (Green et al, 2005).[21] This level reflects the much researched association of age and decreased vitamin B12 status and suggests that the levels that were shown in this study may not be solely metformin associated, but reflect more on the age of the patients involved as well. The prevalence of lowered serum vitamin B12 level in our study in the elderly population (age ≥65) was compared with the population in the Green et al study. It was observed that prevalence of decreased serum vitamin B12 status in metformin treated patients in our study was higher than those in the Green et al study. This comparison must be interpreted with caution as there are other factors that may affect the serum vitamin B12 of these patients, which were not addressed in this study (diet, drug interactions, etc.). Furthermore, as the patients recruited into the Green et al study were non-institutionalised, there may have been a level of malnutrition in regard to nutritional status as has been observed in such populations (McElnay et al, 2012).[22] Monique Nervo et al[23] studied that type 2

diabetes patients who had low vitamin B12 levels were of higher age with the mean of 63.7 ± 11.30. In our study, mean age was 57.3 ± 12.2 who had low vitamin B12 levels.

None of the diabetes patients with normal BMI had vitamin B12 deficiency. Most of the vitamin B12 deficient diabetes patients were overweight forming 52.5% of the total. Among obese patients, 51.3% had vitamin B12 deficiency, though not statistically significant. An association between obesity and poor glycaemic control has been reported. Nagrebetsky et al[24] showed that there is a significant association between lower BMI and lower glycated haemoglobin (HbA1c) concentration, an index of glycaemic control. These reports could explain the observed higher BMI in patients on high dose (>1000 mg/day) of metformin compared with patients on low dose. This observation is not surprising as the relationship between obesity and insulin resistance is well established.[25]

CONCLUSION

The present study has explored the relation between the metformin intake and vitamin B12 deficiency in T2DM-

1. This study has proved that age as well as the duration of diabetes increases the risk of developing vitamin B12 deficiency in T2DM patients taking metformin therapy.
2. Longer duration of diabetes is associated with vitamin B12 deficiency.
3. The higher dose of metformin intake has inverse relation with vitamin B12 levels.
4. Uncontrolled diabetes increases the risk of vitamin B12 deficiency. This study has also showed that vitamin B12 deficient T2DM patients on longer metformin therapy had higher FBS, PPBS and HbA1c levels.
5. Hence, type 2 diabetes patients taking long-term, higher-dose metformin therapy should be screened for vitamin B12 deficiency and should be supplemented with vitamin B12.

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